

The University of Chicago

THE RELATIVE DIGESTIBILITY OF
UNSWEETENED EVAPORATED MILK,
BOILED MILK, AND RAW MILK
BY TRYPSIN IN VITRO

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BIOCHEMISTRY
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ZONJA WALLEN-LAWRENCE

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Reprinted from
AMERICAN JOURNAL OF DISEASES OF CHILDREN
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THE RELATIVE DIGESTIBILITY OF UNSWEETENED EVAPORATED MILK, BOILED MILK AND RAW MILK BY TRYPSIN IN VITRO *

ZONJA WALLEN-LAWRENCE, B.S.

An extensive literature, covering the effect of heat treatment on the organic and inorganic constituents of milk, on enzymes, vitamins, etc., has accumulated during the past forty years.¹ An equally large clinical literature records the observations of workers in this country and abroad with regard to the relative merits of raw and heated milk as artificial food for infants. Mojonnier,² in 1905, reported results of digestion in vitro of raw and heat treated milk by artificial gastric juice which showed that evaporated milk was somewhat more digestible than raw, pasteurized or boiled milk. Hess, Koch and Sennewald³ have studied peptic digestion of cow's milk and the effect on it of various modifications used in infant feeding, including boiling. They also showed that heat treated milk is more digestible by pepsin in vitro than raw milk, and they concluded that the greatest value of all the milk modifications studied by them may lie in the softer broken curd which exposes a large surface to the digestive juices and in the denaturation of the proteins. Clinical experience confirms these results. Abt⁴ stated that clinical observers who have fed raw and boiled milk for an equal number of years are agreed that boiled milk is more readily digested than raw. Marriott and Schoenthal,⁵ after an extensive comparative study of unsweetened evaporated milk and ordinary bottled

* From the Laboratories of Physiological Chemistry, the University of Chicago.

* This work was done during the tenure of a fellowship from the Evaporated Milk Association.

1. Lane-Clayton: *Milk in Its Hygienic Relations*, New York, Longmans Green & Company, 1921.

2. Mojonnier, T.: *The Digestibility of Evaporated Cream*, Med. New. **87**: 877, 1905.

3. Hess, J. H.; Koch, E. M., and Sennewald, Z. C.: *Peptic Digestion of Cow's Milk*, J. A. M. A. **87**:1360 (Oct. 23) 1926.

4. Abt's *Pediatrics*, Philadelphia, W. B. Saunders Company, 1921, vol. 2, p. 675.

5. Marriott, William McKim, and Schoenthal, L.: *An Experimental Study of the Use of Unsweetened Evaporated Milk for the Preparation of Infant Feeding Formulas*, Arch. Pediat. **46**:135, 1929.

milk in the feeding of new-born, well, sick and premature infants, recommended unsweetened evaporated milk and enumerated its advantages over ordinary cow's milk for artificial infant feeding. Brennemann⁶ from his experiments in feeding sick ward babies, without selection of cases, on simple mixtures of evaporated milk and water, stated, ". . . the immediate result of feeding . . . was the most startling I have ever encountered in more than twenty-five years of hospital experience in feeding ward babies." Other physicians have published similar reports. So from laboratory and clinical evidence, it is agreed that heat treated milk is more readily digested than raw.

THE INCREASE IN TRYPSIN DIGESTIBILITY IS NOT CORRELATED
WITH A CHANGE IN THE BUFFER SYSTEM OF MILK

What accounts for this increased digestibility? Since cow's milk is more greatly buffered than human milk,⁷ and since evaporated milk or boiled milk is handled by the infant more nearly like human milk, the question may be asked whether a radical change in the buffer system occurs on treatment with heat. It is our experience that it takes slightly more acid to bring a given volume of evaporated milk (diluted 1:2) to p_H 1.9 than it does to bring an equal volume of raw milk to that p_H , suggesting that evaporated milk is not less buffered than raw milk. There is also evidence that heat treatment does not appreciably alter the concentration of soluble inorganic constituents in milk,⁸ and this is confirmed by observations on electrical conductivity. Rice⁹ has shown that the electrical conductivity of evaporated milk is within the limits ordinarily observed for raw milk. There is also clinical experimentation in progress which may confirm these laboratory observations.⁶ So the conclusion must be drawn that a change in the buffer system adequate to account for the altered digestibility of heat treated milk does not occur.

Brennemann¹⁰ in his classic experiments on coagulation of cow's milk in the human stomach has conclusively shown that boiled and evaporated milks form softer, finer curds in the stomach (as well as in vitro) than does raw milk. He has further shown that milk pre-coagulated with acid, with rennin, or with both, milk treated with lime

6. Brennemann, J.: The Curd and the Buffer in Infant Feeding, J. A. M. A. **92**:364 (Feb. 2) 1929.

7. Marriott, W. M., and Davidson, L. T.: The Acidity of the Gastric Content of Infants, Am. J. Dis. Child. **26**:542 (Dec.) 1923.

8. Palmer, L. S.: The Effect of Heat on the Calcium Salts and Rennet Coagulability of Cow's Milk, Proc. Soc. Exper. Biol. & Med. **19**:137, 1921-1922.

9. Rice, F. E.: The Use of Unsweetened Evaporated Milk in Infant Feeding, Arch. Pediat. **44**:758, 1927.

10. Brennemann, J.: The Coagulation of Milk in the Human Stomach, Arch. Pediat. **34**:81, 1917.

water or magnesia, milk to which barley water is added, and peptonized milk, all have two things in common: they all form a fine curd in the human stomach, and they are universally recognized as more easily digested by both well and ill infants. This raises the question, is the difference in digestibility of raw and heat treated milks a function solely of the nature of the curd?

THE QUESTION OF THE CURD

To throw some light on this question the digestibility, *in vitro*, of evaporated, boiled and pasteurized milks has been compared with that of whole raw milk. Tryptic digestion has been studied, and since this is carried on in an alkaline medium, the complicating factor of difference in size and consistency of the curd does not enter into the work. The criteria of digestibility have been the increase in total soluble nitrogen and the increase in refractometric readings of the protein-free filtrates before and after a period of digestion.

For our earlier studies unsweetened evaporated milk was diluted with distilled water in such quantity that by macro-Kjeldahl analysis the total nitrogen concentration of the diluted milk equalled that of the raw with which it was to be compared. This was so consistently accomplished by a 1:2 dilution of the evaporated milk that ultimately the macro-Kjeldahl control of concentration was dispensed with as unnecessary. When boiled milk was under consideration, a homogeneous sample of whole certified milk was divided into two portions and one portion boiled under a reflux condenser. Whole pasteurized milk was compared with whole certified milk directly, or a given sample of certified milk was divided and part of it pasteurized. Each sample of milk was adjusted electrometrically to p_H 8.2 using dilute sodium carbonate. As soon as the samples had attained a temperature of 38 C., trypsin was added in graduated amounts to each series of flasks, except to the controls. The total nitrogen concentration was kept equal in all flasks by appropriate additions of water, p_H 8.2. Immediately after the addition of the trypsin a sample was removed from each flask and the proteins precipitated from each sample with trichloroacetic acid. The filtrates were analyzed as will be described. The flasks containing milk and trypsin were placed in a vertically rotating shaker in an incubator at 38 C. After four hours of continuous shaking at 38 C., samples were again removed from each flask and the proteins precipitated, as before, with trichloroacetic acid. The water clear filtrates were examined at once in the same way as the "zero hour" filtrates.

In all instances, the filtrates were examined with a Zeiss dipping refractometer at 25 C. The increase in the refractometric reading in the "four hour" filtrate over the reading in the corresponding "zero hour" filtrate was taken as a measure of digestion. That this is war-

ranted has been demonstrated by McMeekin¹¹ for peptic digestion of egg white, and by Helmer¹² for tryptic digestion of casein. However, in the early part of this work the trichloroacetic acid filtrates were also analyzed for total nitrogen by the macro-Kjeldahl method. The data from one such experiment are given in table 1. Chart 1 shows these data plotted, with increases in total nitrogen or increases in refractometer readings as abscissae against increasing trypsin concentration as ordinates. Comparison of the two sets of curves confirms the work of McMeekin and of Helmer, showing that the Kjeldahl method could be dispensed with and that the much more rapid refractometric method could be relied on in subsequent work.

TABLE 1.—*Comparison of the Macro-Kjeldahl and the Refractometric Methods for Measuring Tryptic Digestion*

No.*	Cc. Trypsin	Cc. Water	0 Hr. Refr.	4 Hr. Refr.	Increase	0 Hr. Mg. per cent Nitrogen	4 Hr. Mg. per cent Nitrogen	Increase Mg. per cent Nitrogen
1.....	0	5	51.74	51.75	0.01	26.9	25.4	-1.49
2.....	0	5	51.50	51.50	0.00			
3.....	2	3	51.70	55.40	3.70		124.9	98.0
4.....	2	3	51.68	55.35	3.67		122.8	95.9
5.....	3	2	51.62	56.00	4.98		153.4	126.5
6.....	3	2	51.35	56.71	5.36		167.3	130.4
7.....	4	1	51.39	57.72	6.43		176.8	149.9
8.....	4	1	51.52	57.72	6.20		175.7	148.8
9.....	5	0	51.40	58.62	7.22		192.9	166.0
10.....	5	0	51.24	56.68	7.44		204.4	172.5
11.....	0	5	50.25	50.30	0.05		26.2	0.5
12.....	0	5	50.15	50.22	0.07			
13.....	2	3	50.39	51.40	1.01		61.0	35.4
14.....	2	3	50.69	51.51	0.92		61.4	35.8
15.....	3	2	50.55	53.01	2.46	25.7	99.5	73.9
16.....	3	2	50.54	53.01	2.47		100.7	75.1
17.....	4	1	50.15	54.32	4.17		128.4	102.8
18.....	4	1	50.11	54.02	3.91		125.6	100.0
19.....	5	0	50.18	55.27	5.09		147.0	121.4
20.....	5	0	50.31	55.40	5.09		144.7	119.1

* 1 to 10 inclusive gives data for unsweetened evaporated milk diluted 1:1; 11 to 20 inclusive, for raw certified milk.

Following the method outlined, ten samples of unsweetened evaporated milk, representing five different brands bought in the open market, were compared with whole raw milk. Boiled and pasteurized milks were also compared with raw. The results are illustrated in charts 1 to 4, inclusive. A total of 232 separate comparisons of tryptic digestibility of raw and heat treated milk has been run, and in every case the heat treated milk has been digested more readily than the raw. The degree of change in digestibility appears to be a function of both the temperature to which the milk is heated and the length of time during which it is held at that temperature.

11. McMeekin, T. L.: Studies on the Purification of Pepsin, Thesis (Ph.D.), University of Chicago, 1925.

12. Helmer, O. M.: A Study of Trypsin and Its Activation by Enterokinase, Thesis (Ph.D.), University of Chicago, 1927.

That the physical character of the curd is an important factor in the digestibility of milk by pepsin *in vitro* and in the human stomach cannot be doubted. But these tryptic digestions of raw and heat treated milks also show a marked difference in digestibility even though here the size and consistency of the casein curd have not been a complicating variable. Possibly other differences exist beside the physical character of the curd, which in part may explain the better ease with which milk when boiled or evaporated is handled in digestion.

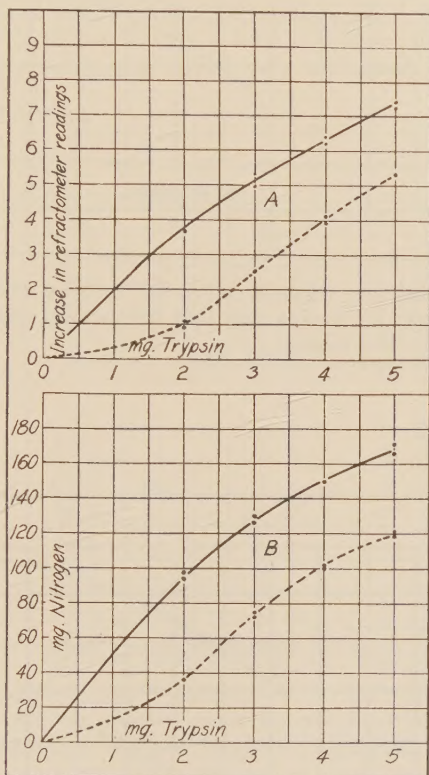


Chart 1.—The solid line indicates curve for evaporated milk; the broken line that for certified raw milk. *A* indicates increases in digestion when using increasing trypsin concentrations and measuring the results refractometrically. *B* indicates the same increase in digestion measured by the increase in total soluble nitrogen expressed in milligrams per hundred cubic centimeters of filtrate. The time of digestion was four hours. For further data see table.

THE DIGESTIBILITY OF THE CASEIN MOLECULE IS NOT ALTERED BY HEAT

It has been suggested that under the influence of heat the casein molecule is changed so as to alter the effect of the digestive enzymes

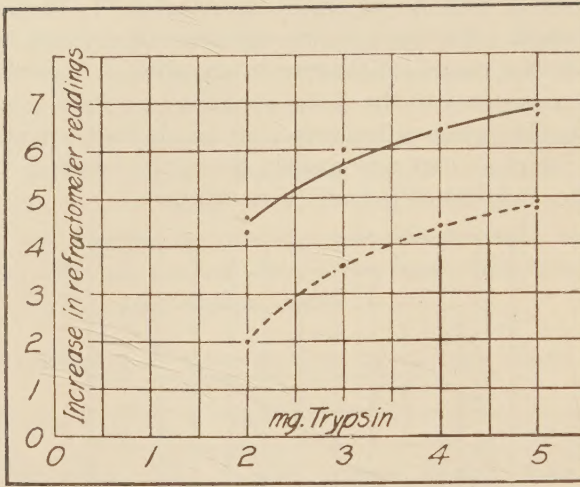


Chart 2.—The solid line indicates curve for certified milk which has been boiled for five minutes; the broken line, that for certified milk not boiled. The time for digestion was four hours.

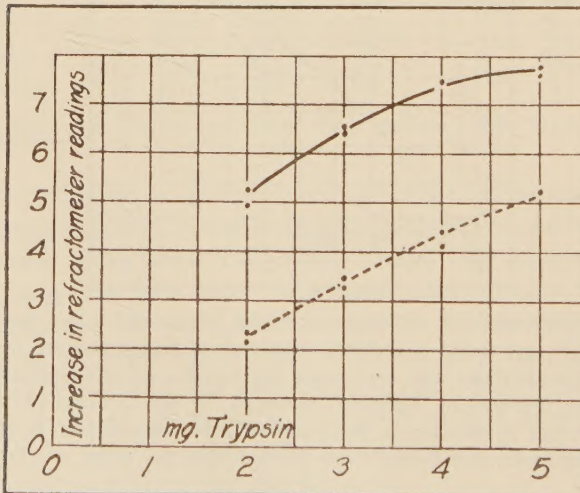


Chart 3.—The solid line indicates curve for milk which has been boiled for ten minutes; the broken line, that for certified milk not boiled. The time for digestion was four hours.

on it. But there seems to be no direct experimental evidence on this point. To confirm or refute the suggestion, the digestibility of raw and heat treated caseins was compared.

Casein for these studies was prepared from whole raw milk (often from skimmed milk) and from evaporated milk by precipitation in the iso-electric range for casein, using approximately molar lactic acid. Lactic acid was used in preference to acetic acid because the latter does not give as good coagulum with undiluted milk.¹³ The acid was added drop by drop while the milk was vigorously agitated with a motor driven stirrer. Under these conditions a fine curd was obtained even from raw milk. The precipitated casein was separated from its whey by

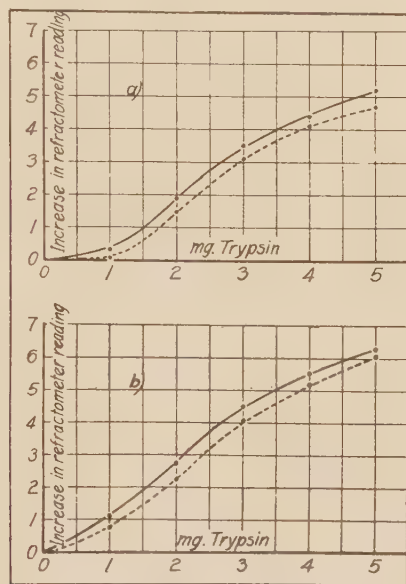


Chart 4.—The solid line indicates curve for pasteurized milk; the broken line, that for raw certified milk. *A* shows results for purchased milk; *B*, for certified milk pasteurized by holding at 60 degrees for thirty minutes. The time for digestion was four hours.

centrifuging. It was then washed ten or twelve times by suspension in water, until the washings were practically free from acid as determined by titration of the total washings. The washed casein was again suspended in water and was dissolved by addition of approximately 5 per cent sodium carbonate solution. This was added drop by drop with vigorous stirring of the casein suspension. In this way casein was readily dissolved without exceeding p_H 7.0. The casein solutions were

13. Tague, E. L.: Casein, Its Preparation, Chemistry and Technical Utilization, New York, Van Nostrand Company, 1926.

used for comparative study after their total nitrogen concentrations were adjusted to equal one another and so as to be approximately the same as that of whole milk. All digestions were made in solutions having an initial p_H of 8.2.

Chart 5 shows the results of an experiment in which the digestibility of a solution of casein preparation from evaporated milk was compared with that of a solution of casein from raw milk. Within the limits of experimental error, there is no demonstrable difference in digestibility. And, furthermore, the casein from raw milk digests more nearly like boiled or evaporated milk than like raw milk. However, it should be pointed out that although the "caseins" were prepared by identical procedures, they probably differ. This is so because the heat

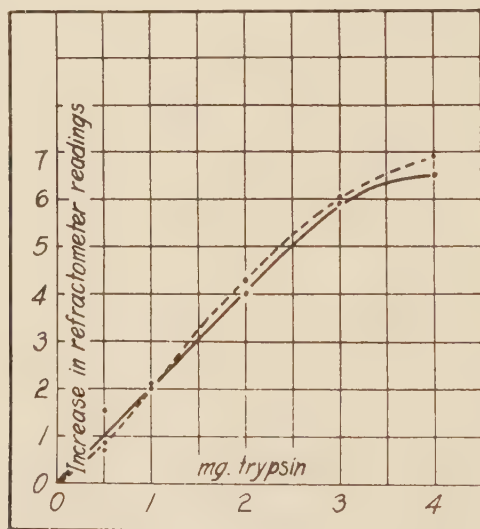


Chart 5.—Curves showing that the proteins precipitated from evaporated milk by lactic acid and thoroughly freed from whey were digested by trypsin, in vitro, at the same rate as the similarly prepared casein from raw milk. The solid line indicates results for casein from evaporated milk; the broken line, for that from raw certified milk. The total nitrogen concentration in each equaled 3.5 per cent protein. The time for digestion was four hours.

coagulated proteins of evaporated milk probably were present to contaminate the casein from evaporated milk. It is possible that a change in digestibility is here masked by the presence of the heat coagulable protein.

This objection was overcome by performing the same experiment with the following variation: casein from certified milk was prepared in the manner already described. The casein was then dissolved in a solution of sodium carbonate to give a solution of p_H 6.5, the p_H of fresh milk. This solution was divided into two equal portions one of

which was boiled for ten minutes. The experimental procedure from then on was exactly as in the previous experiment, and the result of digestion was exactly the same, that is, there was no difference in the rate of digestion of the raw and the heat treated casein. These results have been confirmed further by the use of casein prepared by other methods. Caseins prepared by precipitation with half saturated ammonium sulphate and subsequently dialyzed until sulphate-free, para-caseins from raw and evaporated milks prepared by rennin coagulation, and calcium caseinate, raw and boiled, all show the same thing. There is no difference in the digestibility of raw and heat treated casein by trypsin, and the digestion curves of casein resemble those of evaporated milk.

These results show that in heating no changes take place in the casein molecule which render it more digestible to trypsin. That no radical change takes place on heating casein is also indicated by the work of Cutler¹⁴ using immunologic reactions. He stated that heat

TABLE 2.—*Differences in the Digestibility of Evaporated Milk Whey and Certified Milk Whey*

	Mg. Trypsin	Mg. Nitrogen in 100 Cc. Filtrate	Increase in Soluble Nitrogen in Mg. per 100 Cc. Filtrate
Evaporated milk whey	{ 0.0	21.45	0.0
	{ 0.5	28.73	7.3
	{ 1.0	31.47	10.0
	{ 2.0	32.43	11.0
Certified milk whey	{ 0.0	17.36	0.0
	{ 0.5	18.26	0.9
	{ 1.0	18.92	1.6
	{ 2.0	31.86	14.5

applied to cow's milk in the process of preparing evaporated milk does not appear to change the antigenic capacity of the casein as determined by anaphylactic reactions.

THE CHANGE IN TRYPSIN DIGESTIBILITY IS DUE TO SOME HEAT-LABILE CONSTITUENT OF THE WHEY

These results have thrown suspicion on the whey. Lactic acid wheys, obtained in the course of casein precipitation from certified and evaporated milks were adjusted to p_H 8.2 and digested in the usual manner. When alkalized, each whey gave a precipitate, but the amount of precipitate was noticeably more in the certified whey. This observation was confirmed by macro-Kjeldahl analyses which showed that the certified milk whey contained about twice as much total nitrogen as the other. The increase in soluble nitrogen (nitrogen not precipitated by trichloroacetic acid) in these wheys as a result of digestion is shown in table 2

14. Cutler, O. I.: Antigenic Properties of Evaporated Milk, J. A. M. A. 92:964 (March 23) 1929.

It must be borne in mind that these differences in digestibility are minimal, not maximal. This is due to the fact that the whey from certified milk contained about twice as much total nitrogen as that from evaporated milk. The experiment has been varied by comparing the digestibility of two portions of the same certified whey, one portion of which had been boiled ten minutes. The results were essentially the same. In the experiment, the results of which are given in detail in table 2, the lactate ion concentration may have been slightly different in the two wheys. In the second experiment referred to, there was no difference in the lactate concentration. Since the results of the two experiments are the same, the lactate ion concentration is probably not

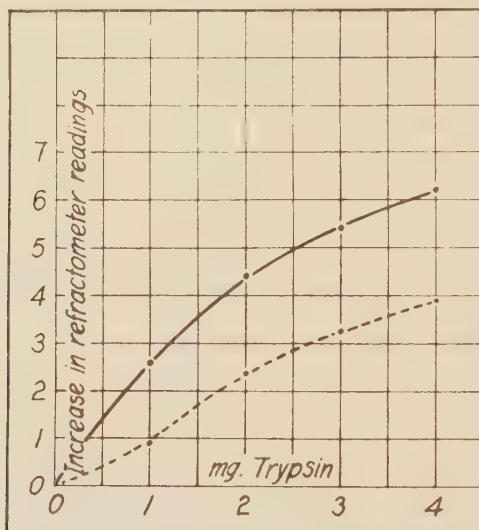


Chart 6.—The solid line indicates results for the digestion of a 3.3 per cent suspension of Hammarsten's casein in water. The initial p_H was 8.2 (no whey). The broken line indicates results for the digestion of a 3.3 per cent suspension of Hammarsten's casein in whey from certified milk. The caseins in this experiment were not so well dispersed into a colloidal state as in most other experiments.

a factor. Obviously, the factor which accounts for the difference in trypsin digestibility of raw and heated milks is in the wheys therefrom.

Further proof of this was obtained by suspending Hammarsten's casein in water and whey from certified milk, respectively, each adjusted to p_H 8.2. The digestion curves for this experiment (chart 6) show that there is something in the whey from certified milk which retards tryptic digestion. This inhibiting factor is heat labile. These observations again are in agreement with those of Cutler¹⁴ who showed that there is an alteration in the antigenic properties of whey protein present

in raw milk when the milk is subjected to the heat necessary to evaporate and sterilize it in the process of manufacture of evaporated milk.

Söldner,¹⁵ in 1888, first called attention to the fact that calcium and phosphorus are precipitated from milk in the ratio of 1 atom of calcium to 1 atom of phosphorus when milk is boiled, and that the amount precipitated varies with the amount and duration of the heat applied. Since the degree of change in the digestibility also appears to be a function of the amount and duration of heating, the question presents itself whether or not calcium and phosphate enter into the explanation of the altered digestibility of milk due to heat.

It seemed simplest to study the phosphate content of the lactic acid whey from certified milk before and after boiling the milk. To clarify the wheys, phosphate-free supercel (hy-flow) was used after it had first been ascertained that supercel does not adsorb phosphate. The method of phosphate analysis used was that of Fiske and Subbarow.¹⁶ The results are listed in table 3. These results are calculated on the

TABLE 3.—*The Phosphate Content of Wheys from Certificate Milk Before and After Boiling the Milk*

Whey from Certified Milk: (Lactic Acid Precipitation)	From Raw Milk, Mg. per Cent	From Milk Boiled Ten Minutes, Mg. per Cent
Total phosphate in clarified whey.....	80.78	80.74
Total phosphate in trichloroacetic acid filtrate.....	80.28	80.28
Inorganic phosphate in trichloroacetic acid filtrate.....	63.52	64.59

basis of undiluted whey. That the results should be identical within the limits of experimental error seemed predictable from the histories of the wheys. Although the phosphate concentration is the same in the wheys from boiled and raw milks, the two wheys digested differently with trypsin, indicating that the phosphate concentration is not a determining factor.

Reasoning from the histories of the two wheys (the identical p_H at which they were prepared and the identical p_H to which they were alkalized before digestion) it seemed unlikely that there could be any difference in the calcium concentration of the two. This, however, was not checked by actual experiment. In the calcium caseinate digestions already referred to, casein was prepared from raw milk in the usual manner except that it was finally dissolved in saturated calcium hydroxide in quantity just sufficient to replace the reported "nondiffusible" calcium of the milk. The p_H of this solution was exactly 7. After adjusting the p_H to that of fresh milk the solution was divided

15. Söldner, F.: Die Salze der Milch und ihre Beziehungen zu dem Verhalten des Kaseins, Landw. Versuchs. **35**:351, 1888.

16. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**:375, 1925.

into two parts one of which was boiled ten minutes. The digestion curves of the raw and boiled calcium caseinates (chart 7) resemble the curves for boiled and evaporated whole milk. From this it again appears that calcium is probably not a factor in the digestibility differences under discussion and that the disturbing factor is in the whey.

This power which raw milk possesses of hindering the action of trypsin is not unique. Hahn,¹⁷ in 1897, showed that normal blood serum possesses a marked resistance against the action of proteolytic enzymes, not being digested at all by dilutions of enzymes that will rapidly digest a serum which has been boiled. This work has been repeatedly confirmed. Wells¹⁸ confirmed it by demonstrating a striking difference in the rate of autolysis of tissues kept in heated and unheated serums, using as his criterion the rate of disappearance of nuclear stain-

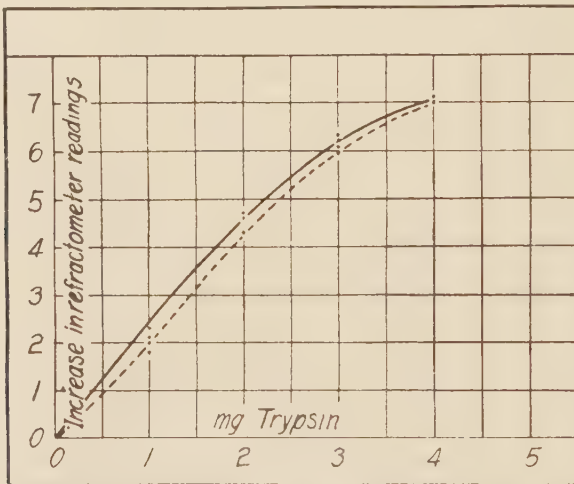


Chart 7.—The solid line indicates curve for calcium caseinate which has been boiled ten minutes; the broken line, that for the same calcium caseinate not boiled. The time for digestion was four hours.

ing. He ascribed the difference to the presence in normal serum of a thermolabile inhibiting substance or anti-enzyme. Efforts have been made by Landsteiner,¹⁹ Glaessner²⁰ and by Cathcart,²¹ to isolate the

17. Hahn, M.: Zur Kenntniss der Wirkungen des extravasculären Blutes, *Klin. Wchnschr.* **39**:499, 1897.

18. Wells, H. G.: The Relation of Autolysis to the Histological Changes Occurring in Necrotic Areas, *J. M. Research* **15**:149, 1906.

19. Landsteiner, K.: Zur Kenntniss der anti-fermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe, *Centralblt. f. Bakt.* **27**:357, 1900.

20. Glaessner, K.: Ueber die antitryptische Wirkung des Blutes, *Hofmeister Beiträge* **4**:79, 1903.

21. Cathcart, E. P.: On the Antitryptic Action of Normal Serum, *J. Physiol.* **31**:497, 1904.

inhibiting substance. Landsteiner and Cathcart agree that the anti-action is found in connection with the albumin fraction. It may well be that in milk we are dealing with a similar, or even the same, "anti-trypsin" which exists in the serum of blood.

Returning to the data shown in table 2, it is important to note that the maximum inhibition of digestion in whey from certified milk takes place in those samples which have the least added trypsin. In fact, this is the relationship which was found in the initial portion of all curves when raw and evaporated milks were compared. The difference in the two curves is established in the course of the action of the first 1.5 to 2 mg. of trypsin; after more trypsin is added, the curves run

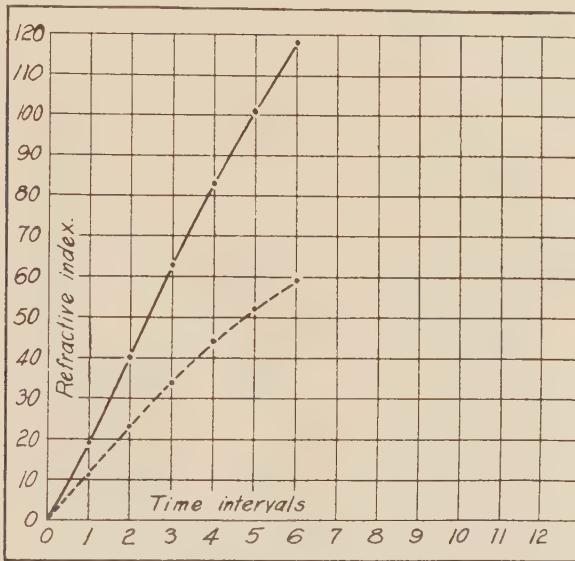


Chart 8.—The solid line indicates that no trypsin inhibitor is present; the broken line, that a trypsin inhibitor is present. The curves were plotted from data calculated on the basis of the following assumptions: that there are 100 molecules of casein and 20 of trypsin per unit volume, that the casein for the sake of convenience is a tetrapeptide, that the trypsin acts catalytically, that one mole of trypsin is decomposed every N minutes (broken curve) and that the refractive index is proportional to the molecular concentration.

parallel. The inference from this was that something other than casein was involved. That inference has been proved correct. With the concept of a tryptic inhibitor in mind it is easy to calculate what effect its presence or its absence should have on the tryptic digestion of casein if the following assumptions are made: 1. Casein is a polypeptide. 2. Trypsin acts catalytically. 3. The inhibiting substance reacts monomolecularly with trypsin rendering it inactive. 4. The refractive index

is proportional to molecular concentration. On these assumptions, data were calculated, and from these data the accompanying charts were drawn.

Chart 8 is based on the assumption that the trypsin concentration is constant and the time variable. Increases in molecular concentration are plotted as abscissae and time intervals as ordinates. This graph prompted the actual carrying out of an experiment in which raw and

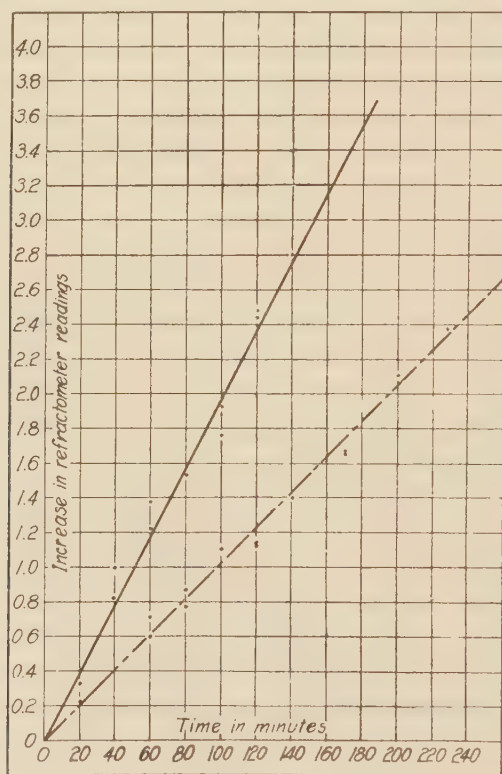


Chart 9.—The solid curve shows results for digestion of evaporated milk; the broken curve, for certified milk. The trypsin concentration is constant.

evaporated milks were digested with a constant quantity of trypsin and time as the variable. The results of this experiment are summarized in chart 9.

Chart 10 sums up the results of the theoretical calculations based on the assumption that trypsin is variable and time constant. These curves show a striking resemblance to the curves for the digestion of raw and evaporated milk. They are not offered as proof of the correctness of the suggestion that we are dealing with an antitrypsin but merely as circumstantial evidence that the antitrypsin hypothesis is not impossible.

THE QUESTION OF THE CURD IS STILL UNSOLVED

Before it can be stated definitely that differences in raw and heat treated milks exist other than those which involve the physical character of the curd, it must be shown what effect pepsin digestion has on the trypsin inhibitor, and the possibility of the presence of a pepsin inhibitor must also be investigated. Only when that is done will this work have practical significance. That work is under way. Probably the "anti-trypsin" destruction in heated milk is not of importance in making such milk better for infants. Very likely the most important factor resides in the peptic phase of digestion and thus probably particularly in the physical character of the curd.

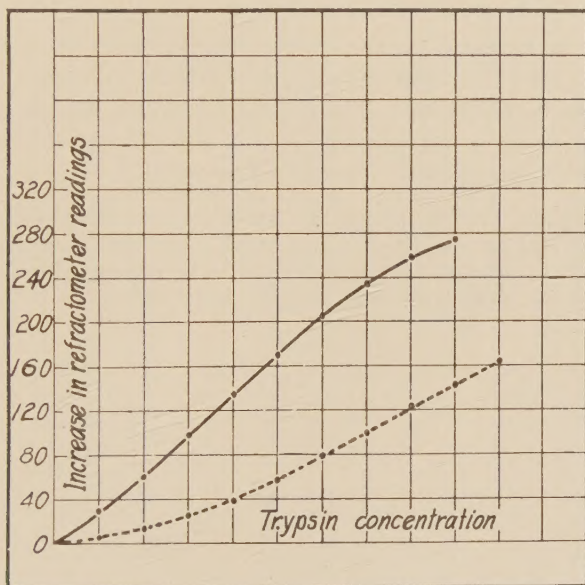


Chart 10.—The solid line indicates curve based on the following assumptions: 100 molecules of tetrapeptide, 10, 20, 20, 40, etc. moles of trypsin, no inhibiting substance and time constant. The broken line indicates curve based on the same assumptions with the addition of: 40 moles of inhibiting substance capable of reacting irreversibly with trypsin. The data in each case have been calculated to the end of 2N time interval so as to get the amino-acid effect on the curve.

SUMMARY

1. Raw milk when pasteurized, boiled or evaporated undergoes changes which make it more readily digested by trypsin *in vitro* than is raw milk. The changes are quantitatively greater for boiled and evaporated milk than for pasteurized milk.

2. The increase in trypsin digestibility appears to be a function of the temperature to which the milk is heated and the length of time it is heated.

3. The increase in trypsin digestibility is not correlated with a change in the buffer system of milk incidental to heating.

4. The increase in trypsin digestibility is not correlated with a change in the casein of the milk.

5. The change in trypsin digestibility is due to some heat-labile constituent of the whey. It is anticipated that this inhibitory substance will be found in the albumin portion of the whey. Calcium and phosphate are probably not involved.

6. It has been assumed that the tryptic inhibitor reacts monomolecularly with trypsin to inactivate it, and on this assumption theoretical curves have been calculated which show a marked resemblance to the curves obtained by actual experiment.

7. It is suggested that no practical significance can be attached to these results until the effect of peptic digestion as a precursor to tryptic digestion has been studied. The question whether the physical nature of the curd alone is responsible for the greater digestibility of heat treated milk in the stomach remains unanswered.

It is a pleasure to acknowledge my indebtedness to Professor F. C. Koch for the kindly interest and valuable suggestions which he has given throughout the work, and to acknowledge gratefully the material aid enjoyed during the incumbency of the Evaporated Milk Association Fellowship.

